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The Study of the Carbohydrate Composition of *Cetraria islandica* (L.) Ach. Thalli Harvested in Ukraine

Abstract

Aim. To study the component composition of free and total monosaccharides in the raw material of *Cetraria islandica* (L.) Ach. harvested in Ukraine.

Materials and methods. The component composition of free and total monosaccharides in the raw material was determined by gas chromatography with mass-spectrometric detection (GC-MS).

Results and discussion. Among the free monosaccharides, the presence of *D*-perseitol, (6.99 mg g^{-1}) and *D*-mannitol (1.12 mg g^{-1}) was determined. Among the total monosaccharides, the content of *D*-glucose (203.64 mg g^{-1}) prevailed. *D*-mannose (53.74 mg g^{-1}), *D*-galactose (51.71 mg g^{-1}), *D*-xylose (0.83 mg g^{-1}) and *L*-rhamnose (0.53 mg g^{-1}) were found in lower quantities, as well as polyatomic alcohols – *D*-dulcitol (8.46 mg g^{-1}) and *D*-mannitol (3.10 mg g^{-1}).

Conclusions. For the first time, the component composition of free and total monosaccharides in the raw material of *C. islandica* harvested in Ukraine has been determined. The results obtained will be used as a component of the comprehensive systematic study of the raw material of *C. islandica* harvested in Ukraine.

Keywords: *Cetraria islandica*; thalli; component composition; free and total monosaccharides; GC-MS

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Вивчення вуглеводного складу слані *Cetraria islandica* (L.) Ach., заготовленої в Україні

Анотація

Мета. Визначити компонентний склад вільних та загальних моносахаридів у сировині *Cetraria islandica* (L.) Ach., заготовленої в Україні.

Матеріали та методи. Визначення компонентного складу вільних та загальних моносахаридів у сировині проводили методом газової хроматографії з мас-спектрометричним детектуванням (ГХ-МС).

Результати і обговорення. Серед вільних моносахаридів виявлено наявність *D*-персеїтолу (6,99 мг $г^{-1}$) та *D*-манітолу (1,12 мг $г^{-1}$). Серед загальних моносахаридів переважав вміст *D*-глюкози (203,64 мг $г^{-1}$). У менших кількостях виявлено *D*-манозу (53,74 мг $г^{-1}$), *D*-галактозу (51,71 мг $г^{-1}$), *D*-ксилозу (0,83 мг $г^{-1}$) і L-рамнозу (0,53 мг $г^{-1}$), а також багатоатомні спирти *D*-дульцитол (8,46 мг $г^{-1}$) і *D*-манітол (3,10 мг $г^{-1}$).

Висновки. Уперше визначено компонентний склад вільних та загальних моносахаридів у сировині *C. islandica*, заготовленої в Україні. Отримані результати буде використано як складову частину комплексного всебічного вивчення сировини *C. islandica*, заготовленої в Україні.

Ключові слова: *Cetraria islandica*; слань; компонентний склад; вільні та загальні моносахариди; ГХ-МС

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Introduction

The study of the carbohydrate composition of plant species that are already used in pharmacy as the medicinal plant raw material does not lose its relevance nowadays [1–3]. In recent years, studies of the pharmacological activity of polysaccharides extracted not only from land plants, but also algae, fungi, and lichens have been conducted [4, 5]. In particular, it has been demonstrated that polysaccharides extracted from green algae exhibit antioxidant, antidiabetic and anti-obesity effects [6]. There are also data on the ability of polysaccharides obtained from various types of fungi to exhibit the antitumor activity [7].

Polysaccharides are one of the main groups of biologically active substances (BAS) of lichens, which attract attention to study various types of the pharmacological activity [8, 9]. The polysaccharide composition was determined only for a small number of lichens, mainly members of the families *Parmeliaceae*, *Cladoniaceae* and *Teloschistaceae* [10]. It has been stated that lichen polysaccharides are not characterized by significant variability of monosaccharides that are part of their structure. *D*-glucose, *D*-galactose and *D*-mannose commonly prevail in different ratios; *L*-rhamnose, *L*-arabinose and *D*-xylose are less common structural units [8, 10]. The main structural forms found in lichens are *β*- and *α*-glucans and *α*-mannans [9].

β-*D*-1,3/1,4-glucan lichenin and α-*D*-1,3/1,4-glucan isolichenin are specific polysaccharides for *Cetraria islandica* (L.) Ach. [11, 12]. According to the data the quantitative content of lichenin can reach up to 27% of the amount of polymeric carbohydrates in a dry substance [13].

The presence of lichenin and isolichenin in the raw material of *C. islandica* is associated with a strong anti-inflammatory and expectorant effect in diseases of the upper respiratory tract, as well as a stimulating effect on various parts of the immune defense system [11, 14, 15]. The recent research was devoted to the study of mechanisms of wound healing and the antitumor action of lichenin, and showed the absence of such activity for lichenin-derived oligosaccharides [16].

Since the extraction of lichenin and isolichenin from the raw material requires special conditions, in particular temperature and pH of an extractant [16, 17], there are studies devoted to the optimization of the method to obtain these and other polysaccharides from *C. islandica* [18–20].

Studies of the carbohydrate composition of *C. islandica* were actively conducted in the late 1990s–early 2000s [9, 13, 20, 21]. The works were related to the isolation, analysis of the structure and the component composition of polysaccharides, in particular, the determination of the monosaccharide ratios [20, 21], as well as the nutritional value of lichen as fodder for reindeer [13]. The presence of *D*-glucose, *D*-galactose, *D*-mannose, *L*-rhamnose, *L*-arabinose, *D*-xylose and *L*-fucose was determined in the raw material of *C. islandica* collected in Norway [13]. In the literature sources available to us, we found a few data on the carbohydrate composition of the raw material of *C. islandica* harvested in Ukraine – polysaccharides in the raw material harvested in the Ivano-Frankivsk region were studied [22].

Therefore, the study of the component composition of monosaccharides in the thalli of *C. islandica* harvested in Ukraine is a relevant issue as a part of the comprehensive systematic study of the component composition of BAS of the raw material and its further processing and use in pharmacy.

Hence, the aim of the work was to study the component composition of free and total monosaccharides in the raw material of *C. islandica* (L.) Ach. harvested in Ukraine.

Materials and methods

Plant raw material

Thalli of *C. islandica* collected in the fall of 2019 in the Rakhiv district of the Zakarpattia region were used for the study. The raw material was dried to an air-dried condition in the open air under a cover and stored in paper bags in a dry place.

Sample preparation. The plant raw material was ground by a laboratory mill LGM-1 (Olis, Ukraine), then 335 mg of the sample was placed in a round-bottom flask and 10 mL of 80% ethyl alcohol; *D*-sorbitol as an internal standard (Sigma-Aldrich, USA) was added (500 μg per sample). Extraction of the free monosaccharides was carried out on a boiling water bath with a reflux condenser for 2 h. After that, 2 mL of the extract was separated, evaporated to remove the solvents in a rotary evaporator and resuspended by adding an aqueous solution of the internal standard (250 μg in 2 mL of water per sample).

To determine the total monosaccharides, 90 mg of the ground raw material was placed in a round-bottomed flask, and 5 mL of 2 M trifluoroacetic

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acid was added. Hydrolysis was carried out on a boiling water bath with a reflux condenser for 6 h. Then 2 mL of the hydrolyzate was separated, evaporated until the solvents were removed, in the process water was added several times, with further removal of the hydrolyzate to complete elimination of trifluoroacetic acid. Re-suspending was carried out by adding an aqueous solution of the internal standard (250 μg in 2 mL of water per sample) [3, 23].

Derivatization. To obtain aldononitrile derivatives of monosaccharides, the pre-treated samples of the extract were taken, and 0.3 mL of the derivatizing reagent (32 mg mL^{-1} of hydroxylamine hydrochloride in a mixture of pyridine/methanol (4:1 *v*/*v*)) was added to each one. The mixture obtained was incubated in an oven at 75°C for 25 min. For acetylation of aldononitrile derivatives of the monosaccharides obtained, 1 mL of acetic anhydride was added, and the mixture was kept in an oven at 75°C for 15 min. Then, 2 mL of dichloroethane was added to the reaction mixture, the excess of derivatization reagents was removed by consecutive processing of the mixture with 1 N hydrochloric acid and water. The dichloroethane layer was dried in a rotary evaporator and dissolved in 300 μL of the mixture of heptane/ethyl acetate (1:1 *v*/*v*) [23].

Conditions of chromatographic separation. The component composition of free and total monosaccharides in the raw material was determined by gas chromatography with mass-spectrometric detection (GC-MS). For the analysis of free and total monosaccharides, aldononitrile acetates were obtained after the appropriate sample preparation [23].

The chromatographic separation was performed on an Agilent 6890N gas chromatograph with a 5973 inert mass detector (Agilent technologies, USA) and a HP-5ms capillary column (30 m × 0.25 mm × 0.25 μm, Agilent technologies, USA). The temperature of the evaporator was 250°C, the temperature of the interface was 280°C. The separation was carried out in the following temperature programming mode: the initial temperature of 160°C was held for 8 min, then raised to 240°C at the rate of 5°C min^{-1} and maintained at this point for 6 min. 1 μL of the sample was injected in the split mode 1:50. Detection was performed in the SCAN mode in the width range of 38–400 m/z. Helium was used as a carrier gas with a flow rate of 1.2 mL min^{-1} [24].

Identification and quantification. Identification of monosaccharides of the mixture studied was based on their retention times compared to standards of monosaccharides (Sigma-Aldrich, USA) and using the mass spectral library NIST 02.

The quantitative content of monosaccharides (mg g^{-1}) was calculated according to the formula:

$$X = \frac{S_x \times M_{inst} \times V_{sol} \times 1000}{S_{inst} \times m \times V_{extr}},$$

where: S_x is the peak area of the compound; S_{inst} is the peak area of the internal standard; M_{inst} is the mass of the internal standard per sample, mg; m is the mass of the sample, mg; V_{sol} is the volume of the solvent for extraction, mL; V_{extr} is the volume of the extract for derivatization, mL [23].

Results and discussion

The chromatogram of the free monosaccharides is shown in Figure 1. The results of the determination of the component composition and the quantitative content of the free monosaccharides in the raw material of *C. islandica*, as well as their chromatographic parameters are shown in Table. 1. The peak with RT 9.77 on the chromatogram corresponds to triacetin that do not belong to the class of BAS studied, and, therefore, is not discussed further.

According to the data in Table 1, sugar alcohols – *D*-perseitol with the content of 6.99 mg g^{-1}, and *D*-mannitol with the content of 1.12 mg g^{-1} were identified.

The chromatogram of the total monosaccharides is shown in Figure 2. The results of determining the component composition and the quantitative content of the total monosaccharides, as well as their chromatographic parameters, are shown in Table 2. The rest of the peaks on the chromatogram correspond to the identified compounds that do not belong to the class BAS studied or have not been identified.

According to the data in Table 2, seven compounds were identified; among them 5 belonged to monosaccharides and 2 – to sugar alcohols. Among monosaccharides, *D*-glucose dominated – 203.64 mg g^{-1}. A high content of *D*-mannose (53.74 mg g^{-1}) and *D*-galactose (51.71 mg g^{-1}) was also determined. Much lower content was observed for *D*-xylose (0.83 mg g^{-1}) and *L*-rhamnose (0.53 mg g^{-1}). The quantitative content of sugar alcohol – *D*-dulcitol (*D*-galactitol) was 8.46 mg g^{-1}, *D*-mannitol – 3.10 mg g^{-1}. The data regarding *D*-dulcitol in the raw material of *C. islandica* was presumably reported for the first time.

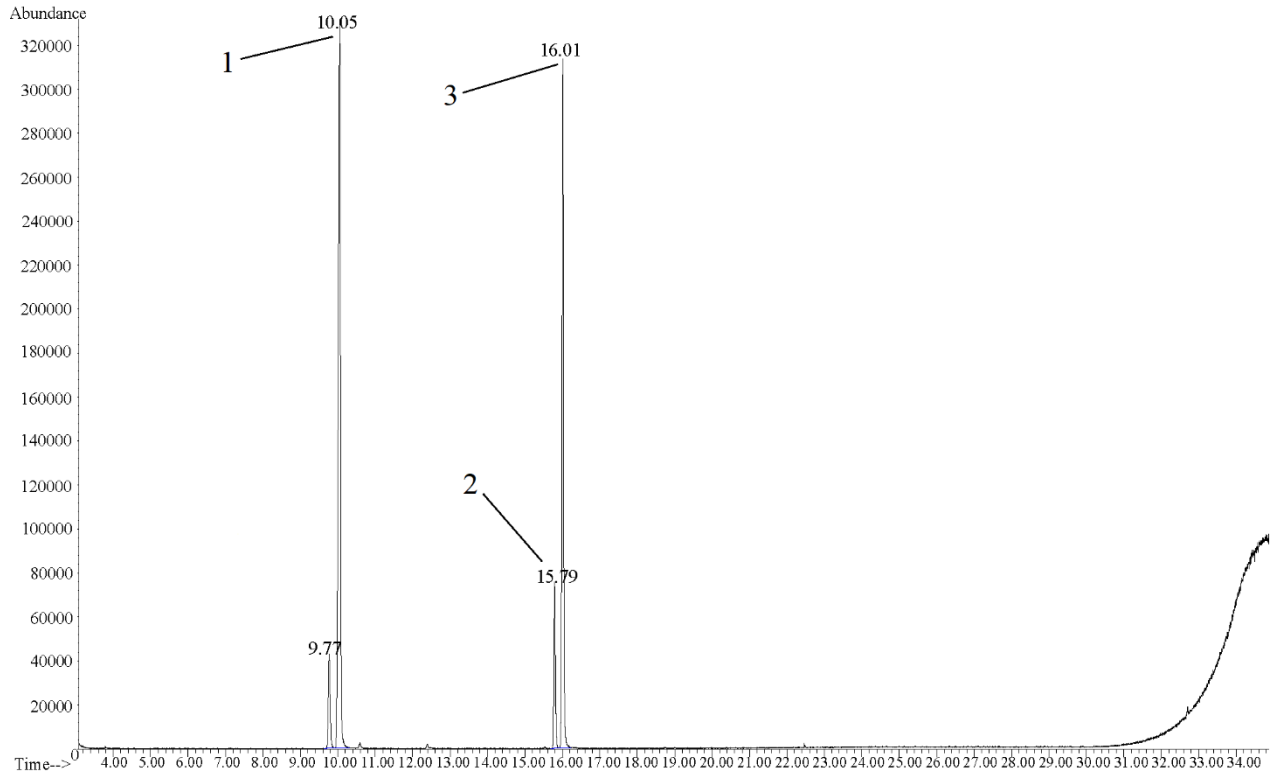

Figure 1. The GC-MS chromatogram of the free monosaccharides in the raw material of *C. islandica*

Table 1. The component composition of the free monosaccharides in the raw material of *C. islandica*

Peak number	Retention time, min	Peak area	Compound	Content, mg g^{-1}
1	10.0503	50.3532	*D*-perseitol	6.99
2	15.792	8.0327	*D*-manitol	1.12
3	16.013	35.8272	*D*-sorbitol	Internal standard

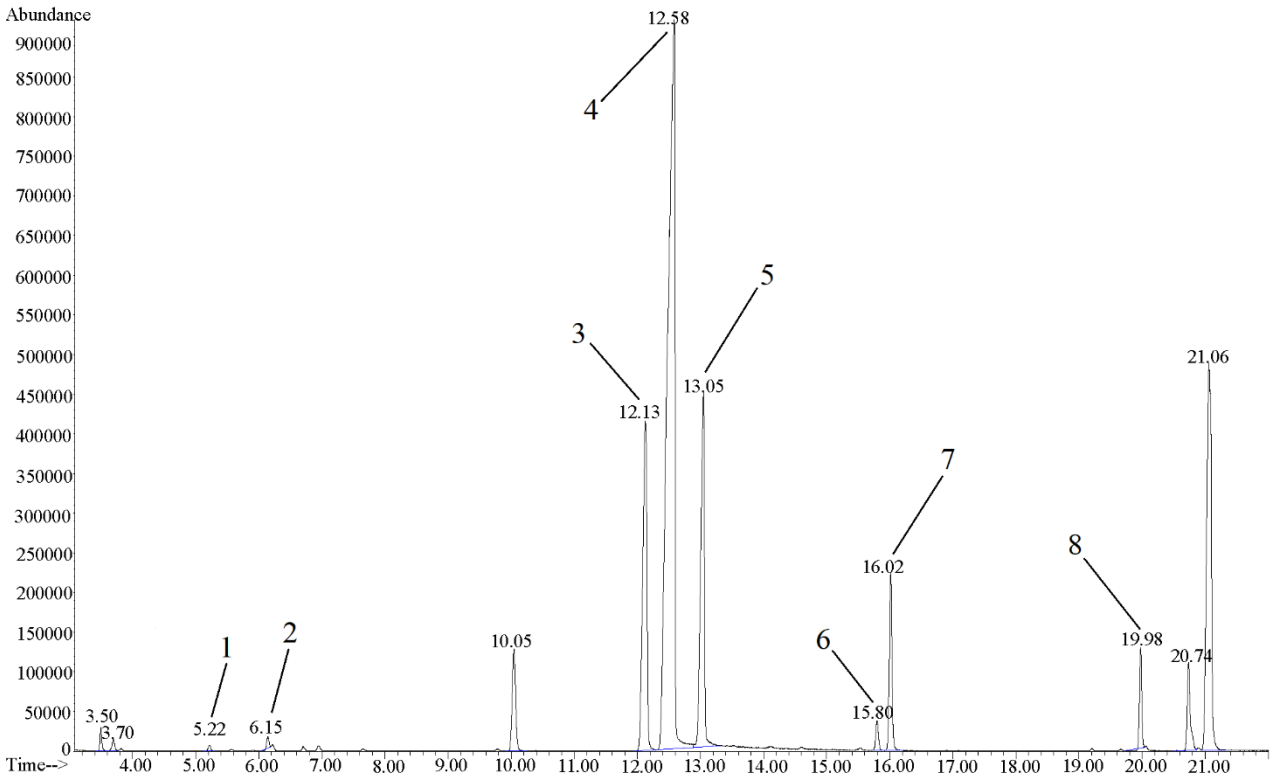

Figure 2. The GC-MS chromatogram of the total monosaccharides in the raw material of *C. islandica*

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Table 2. The component composition of the total monosaccharides in the raw material of *C. islandica*

Peak number	Retention time, min	Peak area	Compound	Content, mg g^{-1}
1	5.2224	0.1217	*L*-rhamnose	0.53
2	6.1447	0.1887	*D*-xylose	0.83
3	12.1286	12.244	D-mannose	53.74
4	12.5834	46.3979	*D*-glucose	203.64
5	13.0466	11.7829	*D*-galactose	51.71
6	15.8006	0.7074	*D*-manitol	3.10
7	16.0173	4.2194	*D*-sorbitol	Internal standard
8	19.9783	1.9287	*D*-Dulcitol	8.46

■ Conclusions

1. For the first time, the component composition of free and total monosaccharides in the raw material of *C. islandica* harvested in Ukraine has been determined.

2. Among the free monosaccharides, the presence of D-mannitol has been determined; its quantitative content is 1.12 mg g^{-1}. Among the total monosaccharides, the content of D-glucose (203.64 mg g^{-1}), D-mannose (53.74 mg g^{-1}) and D-galactose (51.71 mg g^{-1}) prevails.

3. The results obtained will be used as a component of the comprehensive systematic study of the raw material of *C. islandica* harvested in Ukraine; they will be taken into account for further research.

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